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Transmission of Verticillium wilt resistance to tetraploid potato via unilateral sexual polyploidization

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Summary

Verticillium wilt is a serious disease of potato and is caused by the soil-borne fungi *Verticillium dahliae* and *V. albo-atrum*. No major cultivar is resistant to this disease. Two diploid interspecific potato clones, C287 and C545, were previously identified with consistently high levels of Verticillium wilt resistance and are thought to have the same genotype for the loci that confer resistance to *V. dahliae* stem colonization. The purpose of this study was to determine whether resistance to *V. dahliae* stem colonization could be transferred to the tetraploid level in potato via unilateral sexual polyploidization (USP). Progenies in eight families obtained by crossing C287 and C545 to two tetraploid breeding clones, S438 and S440, and the cultivar 'Atlantic' were planted in a *V. dahliae*-infested field and a field without a significant *V. dahliae* infestation. Resistance was evaluated relative to C545 and C287. There were differences among families for stem colonization and yield. No differences between the mean stem colonization of C545 and C287 progeny were detected. Family differences due to the tetraploid parents indicate that they contributed resistance to the progeny. Stem colonization data from this experiment were consistent with the proposed complementary two-gene model for Verticillium wilt resistance in the diploid parents. Unilateral sexual polyploidization is an effective method for transferring *V. dahliae* stem colonization resistance to the tetraploid level.

An important disease of potato (*Solanum tuberosum* L.) is Verticillium wilt (Vw), which causes early plant senescence and yield reductions of 10-50% (Davis 1981, Rouse 1985, Rowe 1985, Rowe et al., 1987). Verticillium wilt is mainly caused by the soil-borne fungi *Verticillium dahliae* and *V. albo-atrum* (Reinke and Barth).

Verticillium wilt resistance is heritable and appears to be stable (Jansky et al., 2004). Several potato cultivars with some resistance to Vw have been released; they include 'Red Dale,' 'Ranger Russet' and 'Defender.' These cultivars have not replaced the most widely grown varieties. Thus, further research and development efforts are required to develop cultivars resistant to Verticillium wilt.

Diploid wild *Solanum* species have shown promise as a potent source of resistance genes to Vw (Concibido et al., 1994, Corsini et al., 1988, Lynch et al., 1997). Wild *Solanum* species typically do not tuberize under the long photoperiod of a temperate summer. However, they can be crossed to dihaploids ($2n = 2x = 24$) of *S. tuberosum*, producing vigorous hybrids that will tuberize during the growing season (Hermundstad & Peloquin 1986, Hougas & Peloquin 1960).

Two diploid interspecific potato clones (derived from haploid-species hybrids) have been identified with consistently high levels of Vw resistance (Jansky et al., 2004, Jansky & Rouse 2000, Jansky & Rouse 2003). Two major dominant genes for resistance appear to be operating in these clones (Jansky et al., 2004). A

Table 1. Pedigrees of families and numbers of clones evaluated in 2002 and 2003

Family	Female parent	Male parent	2002	2003
1	S438	C287	15	12
2	S440	C287	34	28
3	C545	S438	21	18
4	C545	S440	8	8
5	S438	C545	5	5
6	S440	C545	35	35
7	Atlantic	C287	10	10
8	Atlantic	C545	14	13

strategy to transfer this resistance to the tetraploid level is unilateral sexual polyploidization (USP) (Mendiburu & Peloquin 1976, Ortiz 1998), wherein the resistant diploids are crossed to tetraploid clones and, if 2*n* gametes are present in the diploids, tetraploid offspring are produced. This scheme has been used to transfer to the tetraploid level resistance to root knot nematodes (Iwanaga et al., 1989), bacterial wilt (Watanabe et al., 1992), early blight (Herriott et al., 1990), and common scab (Murphy et al., 1995).

The objective of this research was to determine whether resistance to Vw is effectively transferred to tetraploid clones following USP.

Materials and methods

Pedigrees

The two diploid Vw resistant clones used in this study were:

C287 [US-730 × (*S. berthaultii* × *S. tarijense*)] × [US-W730 × *S. tarijense*]

C545 [H551 × *S. chacoense*] × [US-730 × (*S. berthaultii* × *S. tarijense*)].

US-W730 is a dihaploid derived from the tetraploid breeding clone Wis Ag 231, while H551 is a dihaploid of unknown parentage. Neither resistant clone has detectable levels of 2*n* pollen based on visual screening for large pollen grains. However, both clones were crossed repeatedly as males to 'Atlantic' and as both males and females to the breeding clones S438 and S440. S438 and S440 are full-sibs from a cross between Wis Ag 231 as a female and a US-W2838 ('Merrimack' haploid)-*S. tarijense* hybrid. Eight families were obtained (Table 1) and planted in the field at the Hancock, Wisconsin, Agricultural Experiment Station to obtain tubers in 2000. A seed increase was carried out in 2001 by planting four hills per clone.

2002

In 2002, 142 clones from the eight $4x \times 2x$ and $2x \times 4x$ families were planted in three replications of four-hill units on each of two fields in a randomized complete block design (RCBD). The first field, planted on 30 April, is the Vw screening field containing *V. dahliae.* This field has been planted continuously with potato for over 30 years and contains an average of 6.5 colony-forming units (CFU) of *V. dahliae* per gram of soil. The second field, planted on 2 May, had no significant *V. dahliae* infestation. On both fields, emergence notes were taken on 5 June. Clones on the Vw screening field were scored for disease on 15, 29 July and 12, 26 August by estimating the percent foliage with wilt symptoms per plot. Plants were allowed to senesce naturally and then apical mainstem segments were collected from two plants in each plot in the Vw field in September. All stems from a plant were combined and ground in a Wiley mill with a 40 mesh screen. Then a 25 mg sample was plated on NPX medium (Butterfield & DeVay 1977) and incubated at room temperature in the dark. After two weeks, the stem material was washed from the plates and the number of colonies of *V. dahliae* per plate was counted. All plots were harvested on 4 October and tuber yield was determined. Yield loss was calculated for a clone by the following formula.

$$\text{Yield loss}\,(\%) = 100 \times \left(1 - \frac{(\text{yield from infested plots})}{(\text{yield from uninfested plots})}\right)$$

2003

In 2003, 129 clones were planted on 2 May in the same manner as described in 2002. Thirteen of the original 142 clones did not yield enough seed in 2002 to be included in the study in 2003. Emergence was recorded on 3 June. Clones on the Vw screening field were scored for disease on 9, 16, 23 and 30 July by estimating the percent wilt symptoms. Plants were allowed to senesce in the field. Apical mainstem segments were collected from one plant in each plot in September. The apical stems from each plant were ground in a Wiley mill with a 40 mesh screen. A 25 mg sample of each ground stem was plated on NPX medium and incubated for two weeks at room temperature without light. The stem

Table 2. Grouped[1] means and standard deviations () for stem colonization, AUDPC, yield loss, and yield of parents in 2002 and 2003

	Clone	Stem colonization[2]	AUDPC[3,4]	Yield loss	Yield (kg)
2002	'Atlantic'	3.74 (1.65)^{b,c}	2537 (283)^{b}	44.3	6.17 (1.67)^{b}
	S438[5]	–	–	–	–
	S440	3.85 (0.41)^{b,c}	2816 (261)^{b}	51.8	5.33 (1.32)^{b}
	C287	1.75 (1.88)^{a,b}	3428 (440)^{c}	21.6	1.42 (0.42)^{a}
	C545	2.96 (1.33)^{a,b,c}	2089 (409)^{a}	2.7	0.28 (0.11)^{a}
2003	'Atlantic'	3.80 (0.62)^{b}	1375 (90)^{a}	31.1	7.57 (1.77)^{c}
	S438	2.93 (0.63)^{b}	1406 (108)^{a}	36.7	5.08 (1.57)^{b}
	S440	3.82 (0.42)^{b}	1585 (111)^{b}	40.9	9.08 (1.00)^{c}
	C287	1.01 (1.53)^{a}	1339 (28)^{a}	59.0	1.05 (0.47)^{a}
	C545	0.00 (0.00)^{a}	1350 (1.4)^{a}	94.1	1.68 (1.61)^{a}

[1]Means within a year separated by pairwise comparisons (Bonferroni; $P = 0.05$).
[2]Log(CFU/g dried apical stem material + 1).
[3]Area under the disease progress curve based on Vw symptom expression.
[4]Disease was assessed more frequently and for a longer duration in 2003.
[5]Seed not available in 2002.

material was washed from the plates and the number of colonies of *V. dahliae* was counted.

Statistical analysis

An analysis of variance (ANOVA) was conducted for each year and combined across years for AUDPC, transformed (log $x + 1$) colony count data and yield loss. The R environment was used for all ANOVA's (R-Development-Core-Team 2004). Means were separated using a pairwise *T*-test (Bonferroni). However, due to moderate (and different) sample sizes, individual clone means were separated using protected *T*-tests at $\alpha = 0.05$. Clones were considered to be resistant if they were significantly more ($\alpha = 0.1$) resistant than or at par with the resistant parent.

Results

Parents

When used as a female parent in the $2x \times 4x$ crosses, C287 did not produce progeny. On the other hand, C545 produced progeny as a female parent, so it must produce $2n$ eggs. Both clones produced offspring as male parents so they apparently produce $2n$ pollen, but at a level below that detectable by the pollen screen.

Within a year, analysis of variance indicated significant differences among parents for yield, AUDPC, and stem colonization (Table 2). In both years C287 and C545 had lower colony counts and yielded less than the susceptible parents. In 2002, C287 had a larger and C545 had a smaller AUDPC than the susceptible parents. AUDPC values for C287 and C545 were significantly different from each other in 2002, but not in 2003. Also in 2002, C545 had higher colony counts, significantly lower amounts of disease and lower yield than C287. However, in 2003, C545 and C287 did not differ in colony counts or disease.

Analysis of variance over years also indicated significant differences among parents for AUDPC (data not shown). C287 and C545 had significantly ($p < 0.001$) less disease and lower colony counts, in 2003 than in 2002 (Table 2; between-season test not shown). Yield loss for C287 and C545 was higher in 2003 than in 2002.

Progeny (grouped by parent)

In 2002 and 2003, there were no differences between C287 progeny and C545 progeny for stem colonization, disease, yield loss or yield (Table 3). Although the susceptible tetraploid parents ('Atlantic', S438 and S440) had similar amounts of stem colonization, progeny means were significantly different ($P < 0.05$) between years (Table 4). Progeny mean yields among the susceptible tetraploid parents were also significantly ($P < 0.05$) different. 'Atlantic' progeny, on average, yielded higher and exhibited less yield loss than S438 progeny and S440 progeny.

The susceptible $\times$ C545 offspring had lower colony counts than the offspring from the reciprocal cross (data not shown). The difference was significant ($P < 0.01$)

Table 3. Grouped[1] means and standard deviations () for stem colonization, AUDPC, yield loss, and yield of progeny from diploid parents in 2002 and 2003

	Parent	Stem colonization[2]	AUDPC[3,4]	Yield loss	Yield (kg)
2002	C287	3.37 (1.09)[a]	2626 (694)[a]	44 (26)[a]	3.34 (2.18)[a]
	C545	3.36 (1.17)[a]	2601 (825)[a]	49 (39)[a]	3.21 (2.37)[a]
2003	C287	3.00 (1.20)[a]	1372 (343)[a]	37 (30)[a]	6.81 (3.98)[a]
	C545	2.91 (1.28)[a]	1294 (438)[a]	40 (20)[a]	6.27 (3.61)[a]

[1]Means within a year separated by pairwise comparisons (Bonferroni; $P = 0.05$).
[2]Log(CFU/g dried apical stem material + 1).
[3]Area under the disease progress curve based on Vw symptom expression.
[4]Disease was assessed more frequently and for a longer duration in 2003.

Table 4. Grouped[1] means and standard deviations () for stem colonization, AUDPC, yield loss, and yield of progeny from tetraploid parents in 2002 and 2003

	Parent	Stem colonization[2]	AUDPC[3,4]	Yield loss	Yield (kg)
2002	'Atlantic'	3.71 (0.98)[a]	2594 (785)[ab]	36 (35)[a]	5.45 (2.69)[a]
	S440	3.41 (1.10)[a]	2687 (658)[bc]	45 (36)[a]	3.09 (1.93)[b]
	S438	3.15 (1.24)[b]	2476 (933)[a]	55 (30)[a]	2.41 (1.96)[c]
2003	'Atlantic'	3.36 (0.92)[a]	1411 (327)[bc]	35 (17)[a]	8.80 (3.97)[a]
	S440	2.84 (1.30)[b]	1342 (396)[ab]	36 (25)[a]	6.31 (3.56)[b]
	S438	2.86 (1.30)[b]	1223 (458)[a]	46 (27)[a]	5.18 (3.26)[c]

[1]Means within a year separated by pairwise comparisons (Bonferroni; $P = 0.05$).
[2]Log(CFU/g dried apical stem material + 1).
[3]Area under the disease progress curve based on Vw symptom expression.
[4]Disease was assessed more frequently and for a longer duration in 2003.

in 2003, but not in 2002. Also, susceptible × C545 offspring had significantly ($P < 0.05$) higher yields than the reciprocal cross in 2002, but not in 2003. Offspring from Sus. × C545 and the reciprocal cross did not express differences in AUDPC.

Progeny (by family)

Analysis of variance indicated significant ($P < 0.05$) differences among families within a year based on AUDPC, and stem colonization, but not yield loss, in both years (data not shown). Across all families in 2002, a single clone was resistant as measured by the three criteria, stem colonization, AUDPC, and yield loss. Twenty-one clones were resistant based on all three criteria in 2003. Based on data from 2003, S438, when used as a parent, yielded a higher proportion of progeny resistant by all three criteria than S440 or 'Atlantic.' Performance and ranking of a clone based on its mean stem colonization was variable between years and depended greatly on the performance of the parent (Figure 1).

Discussion

Families were created following $4x - 2x$ crosses using Verticillium wilt resistant diploid parents. Because a strong triploid block exists in potato (Marks, 1966), these families are expected to be tetraploid. Clones from these families have been used in subsequent crosses (not reported here), supporting the premise that they are tetraploid rather than triploid.

Verticillium wilt resistance was effectively transferred to the tetraploid level via USP. In the small families generated by this study, clones with high levels of resistance were produced. Stem colonization data on two of these clones, along with the resistant parents and a susceptible cultivar, are presented in Figure 2. Resistance in these clones, based on stem colonization, is comparable to that of the resistant diploid parents.

Although the resistant diploid parents did not produce $2n$ gametes at detectable levels, tetraploid families were produced when large numbers of crosses were made to tetraploid clones. Therefore, the USP scheme can be used even with diploid clones that do not express meiotic mutations for $2n$ gamete production.

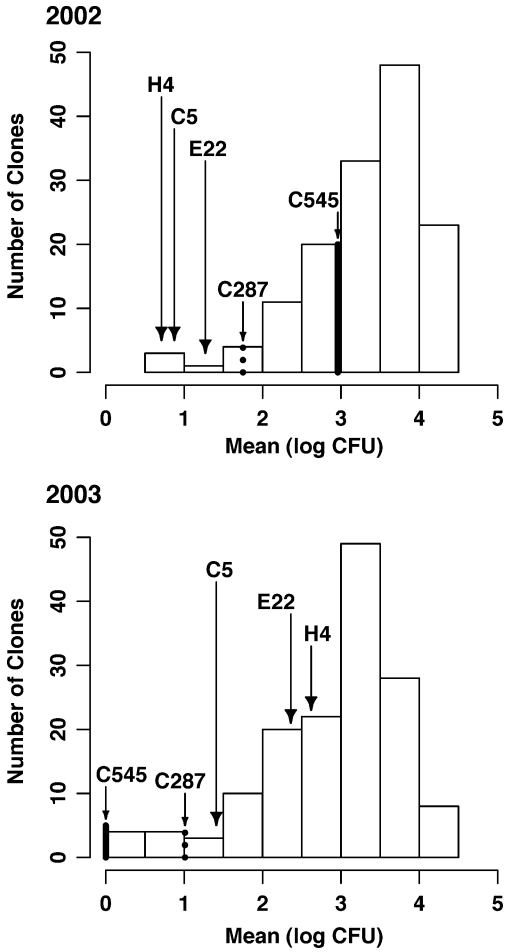

Figure 1. Histograms of the mean stem colonization scores of all clones in 2002 and 2003. Clones C545 and C287 are resistant parents and clones C5, H4, and E22 are 4*x* progeny that exhibit resistance to *V. dahliae* infection.

The production of clones with high levels of resistance in small families supports the hypothesis that major genes are responsible for resistance in the diploid parents. Each diploid is putatively homozygous for one dominant resistance gene and heterozygous for the other (Jansky et al., 2004). Therefore, all tetraploid offspring should carry both copies of the gene that was in the homozygous condition in the diploid parent. However, it is apparent that not all tetraploid clones carry the dominant form of the second resistance gene, since not all are resistant. The 2*n* gametes would carry both recessive copies of that gene if they were formed by a first division restitution mechanism and a crossover occurred between the gene and the centromere. Alternatively, homozygous recessive gametes would result from second division restitution without a crossover between the gene and the centromere. We do not know how 2n gametes were formed during USP in this study. In fact, both types of mechanisms could have contributed to the production of tetraploid offspring. As a result, we can not propose a genetic model against which we can test the tetraploid offspring.

Because there were differences in resistance among families based on the tetraploid parents, they must have contributed some Vw resistance. While most of the resistance to *V. dahliae* stem colonization may be simply inherited from the diploid parents, some may be due to minor genes. Several studies indicate that some minor additive genetic factors in tetraploid susceptible potato clones may contribute to resistance expression (Akeley et al., 1956, Hunter et al., 1968, McLean 1955). Based on this observation, we might be able to select among tetraploid potato clones for those that contribute minor genes for resistance. Combining ability analysis of the tetraploid parents could be performed by crossing to a common diploid parent and determining which produces resistant progeny at the highest frequency. There is some evidence that combining ability is an important feature of the expression of certain disease resistances (Tung et al., 1990).

In three out of four comparisons, C545 as a female parent produced progeny that had lower colony counts than the reciprocal cross. This suggests that there might be a female by male genotype interaction or that there is a cytoplasmic component to resistance. Reciprocal cross differences have been reported for other traits in potato (DeJong et al., 2001, Sanford & Hanneman 1979). However, the significance of the reciprocal difference in this study is not known. It is unfortunate that C287 did not produce progeny when used as a female parent so that we could further evaluate this observation.

Among the methods used in this study, stem colonization appeared to most effectively identify resistance to VW. Although within clone stem-to-stem CFU variability was observed in both years (data not presented), this did not obscure our ability to identify the most resistant clones (Figure 2). On the other hand, yield loss data were sometimes highly variable as a result of the small plot size. Errors made when measuring yields from small plots might be exaggerated in the yield loss calculation. This is especially true for low yielding clones such as C545. High AUDPC scores were observed for the Vw resistant parents and some of their offspring. These may be due, in part, to mineral toxicity. C287 and C545 are known to hyperaccumulate

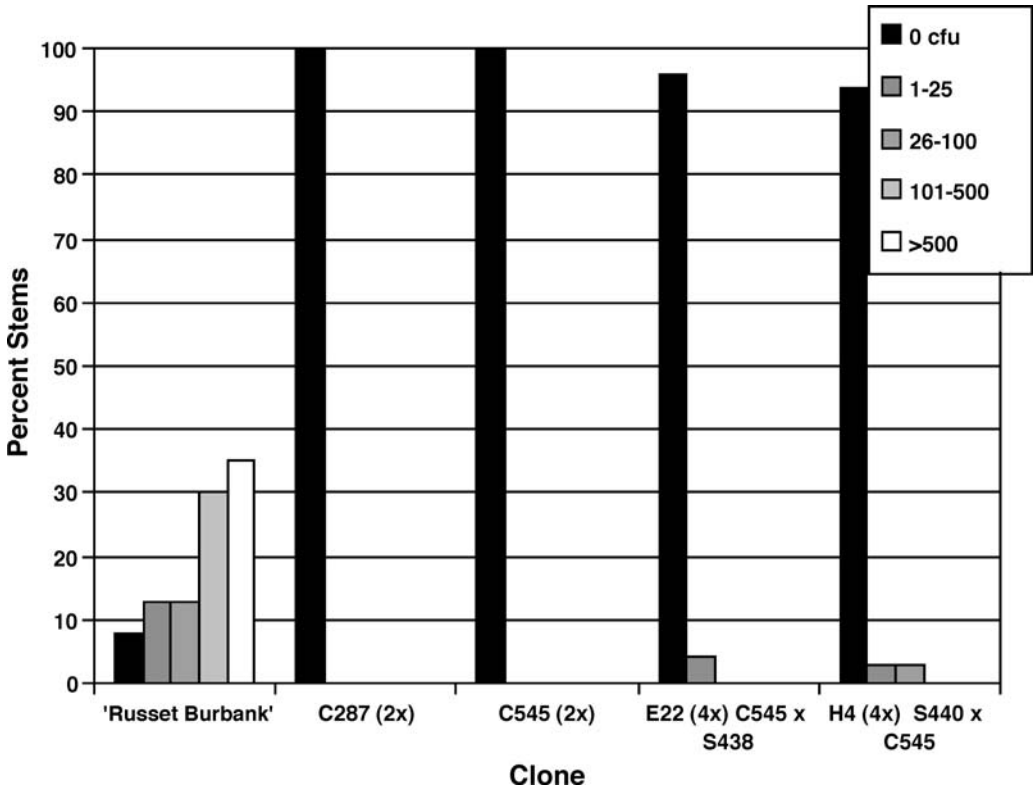

Figure 2. Stem colonization scores in the susceptible cultivar 'Russet Burbank,' two resistant diploid hybrids (C287 and C545) and two clones created by USP (E22 and H4). Each bar represents the percent stems with the indicated number of colony forming units per 100 ul stem sap.

certain divalent cations (data not presented). They may also be expressing symptoms of other diseases, such as early blight. The inability to completely distinguish between Vw symptoms and other physiological or disease problems highlights a limitation of using visual disease data alone for Vw resistance screening.

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